ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 10, Issue 11, 2017

Online - 2455-3891
Print - 0974-2441

Research Article

IN VITRO ANTIOXIDANT AND ANTIHYPERGLYCEMIC ACTIVITIES OF HYDROETHANOLIC LEAF EXTRACT OF *ACACIA CATECHU* (L.F) WILLD

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Received: 29 May 2017, Revised and Accepted: 24 July 2017

ABSTRACT

Objective: The aim of this study was to analyze the antioxidant and antihyperglycemic activities of hydroethanolic leaf extract of *Acacia catechu* (L.f) Willd.

Methods: The *A. catechu* (L.f) Willd. leaf extract was subjected to antioxidant activities such as 1,1-diphenyl-1-picryl hydrazyl (DPPH), hydroxyl radical (OH^-), nitric oxide radical (NO^-), and superoxide radical (SO) scavenging assays. Further, the leaf extract was subjected to antihyperglycemic activity by α-amylase inhibition assay.

Results: The free radical scavenging activity of the plant extract was found to increase with increase in concentration (20-100 µg/ml) and the maximum activity was obtained at 100 µg/ml, which was compared with the standard ascorbic acid. The percentage inhibition of free radicals was found to be 62.94±0.91%, 63.53±1.08%, 71.04±0.48%, and 51.50±0.86% for DPPH, OH^-, NO^-, and SO^-, respectively. The IC_{50} for DPPH, OH^-, NO^-, and SO were recorded as 73.81±0.95, 69.03±0.58, 60.74±0.37, and 83.10±1.00 µg/ml. The IC_{50} value of α-amylase inhibitory activity was found to be 71.17 µg/ml.

Conclusion: The present investigations suggest that the *A. catechu* (L.f) Willd may serve as a potential source of natural antioxidant and antihyperglycemic agent that could have great importance in pharmaceutical preparations.

Keywords: *Acacia catechu* (L.f) Willd, 1,1-diphenyl-1-picryl hydrazyl, Hydroxyl radical, Nitric oxide radical, Superoxide oxide radical, α-amylase inhibition.

 DOI: http://dx.doi.org/10.22159/ajpcr.2017.v10i11.20299

INTRODUCTION

Free radicals are highly reactive and oxygen-containing molecules. Various types of reactive oxygen species include hydroxyl radical (OH^-), superoxide anion radical, singlet oxygen, nitric oxide radical (NO^-), hypochlorite radical, and hydrogen peroxides [1]. Free radicals are produced by physiological/biochemical reactions or by pollution and other endogenous sources. Free radicals react with membrane lipids, proteins, nucleic acids, enzymes, and other small molecules, resulting in cellular damage [2]. The human system is protected by antioxidants, which play an important role in scavenging free radicals, and providing protection against degenerative diseases [3]. In recent years, the research is focused on natural antioxidants from medicinal plants to know their constituents and biological activity [4]. Plants are the rich source of alkaloids, phenolic acids, tannins, flavonoids, terpenoids, and other metabolites that act as primary antioxidants or free radical scavengers [5]. The imbalance between the production of reactive oxygen species and antioxidant defenses leads to oxidative stress [6]. Redox stress cause immune cells to release proinflammatory cytokines, reactive oxygen, and nitrogen species, that damages biological molecules and induce imbalances in physiological and pathological pathways [7]. α-amylase is involved in the breakdown of long chain carbohydrates, serve as the major digestive enzyme and help in intestinal absorption. α-amylase inhibitors are the potential targets for the development of lead compounds for the treatment of diabetes [8].

Acacia catechu (L.f) Willd is a moderate-sized tree, which belongs to the family of *Leguminosae*. It is commonly used in herbal preparations for diabetes [9]. Conventionally, *A. catechu* has been used as an antioxidant, anti-inflammatory, antimicrobial as well as antifungal property and also to treat diabetes and obesity [10]. Natural α-amylase inhibitors from the dietary plants can be used as an effective therapy for treating post-prandial hyperglycemia with minimal side effects. The present study was carried out to investigate the antioxidant activity and inhibitory potential of *A. catechu* (L.f) Willd leaf extract on α-amylase, *in vitro*. Hence, an attempt was made to find remedy for a prevalent clinical ailment through natural source.

METHODS

Plant collection and preparation of extract

The plant *A. catechu* (L.f) Willd was collected from Kanjikode, Kerala, identified and certified by a taxonomist at Botanical Survey of India (BSI), Tamil Nadu Agricultural University, Coimbatore, (Plant Identification No. BSI/SRC/5/23/2014-2015/Tech/699). The leaves of *A. catechu* (L.f) Willd were shade dried and ground to a coarse powder by a mechanical device. The extract was prepared using different solvents, namely, petroleum ether, chloroform, acetone, ethanol, hydroethanol, and water by cold maceration process. The filtrate was used for preliminary phytochemical analysis. Further studies were carried out using the 50% hydroethanolic extract, prepared using soxhlet apparatus. The extract was condensed to dryness using rotary evaporator and the crude residue obtained (15% w/w) was stored in an airtight container until use.

Free radical scavenging assays

1,1-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging assay

DPPH radical scavenging activity was assayed using the method of Brand-Williams *et al.* [11]. The radical scavenging activity of sample against DPPH was determined spectrophotometrically. The reaction mixture in a total volume of 3.0 ml contained 1.0 ml of DPPH, 0.5 ml of sample and made up to 3.0 ml with water. The tubes were incubated for 10 minutes at 37°C. A blue chromophore was formed, the absorbance of which was measured at 515 nm.

OH^- scavenging assay

OH^- scavenging assay was measured by the method of Halliwell *et al.* [12]. The incubation mixture in a total volume of 1.0 ml contained 0.1 ml of buffer, 0.5 ml of sample, 0.2 ml of ferric chloride, 0.1 ml of ascorbic acid, 0.1 ml of ethylenediaminetetraacetic acid (EDTA), 0.1 ml of hydrogen peroxide, and 0.2 ml of 2-deoxyribose. The contents were mixed thoroughly and incubated at room temperature for 60 minutes, and then, added 1.0 ml of thiobarbituric acid and 1.0 ml of trichloroacetic acid. All the tubes were kept in a boiling water bath for 30 minutes. The absorbance of the supernatant was read in a spectrophotometer at 535 nm with reagent blank containing water in place of the extract.

NO^- scavenging assay

NO^- scavenging activity was measured by the method of Sreejayan and Rao [13]. The reaction mixture (3.0 ml) containing 2.0 ml of sodium nitroprusside (10 mM), 0.5 ml of phosphate buffer saline (1.0 M), and 0.5 ml of different concentrations (20-100 µg/ml) of plant extract was incubated at 25°C for 150 minutes. After incubation, 0.5 ml of the Griess reagent was added. Control experiments without the test compound, but with an equal volume of buffer were added. The absorbance of the chromophore formed during diazotization coupling with naphthyl ethylenediamine was read at 540 nm.

Superoxide radical (SO) scavenging assay

The SO scavenging activity was studied using the method of Winterbourn *et al.* [14]. The assay mixture contained (0.2-1.0 ml) sample with 0.1 ml of Nitroblue tetrazolium (1.5 mM NBT) solution, 0.2 ml of EDTA (0.1 mM EDTA), 0.5 ml riboflavin (0.12 mM), and 2.55 ml of phosphate buffer (0.67 M). The control tubes were also setup, wherein dimethyl sulfoxide was added instead of the sample. The reaction mixture was illuminated for 30 minutes and the absorbance at 560 nm was measured against the control sample. All the tests were performed in triplicate and the results averaged. The percentage inhibition was calculated by comparing the results of control and test samples using the formula:

$$\text{Percentage inhibition} = \frac{\text{Absorbance(control)} - \text{Absorbance(sample)}}{\text{Absorbance(control)}} \times 100$$

α-amylase inhibition assay

α-amylase inhibition was studied using the method of Narkhede *et al.* [15]. The enzyme solution was prepared by dissolving α-amylase in 20 mM phosphate buffer (pH=6.9) at the concentration of 0.5 mg/ml. 1.0 ml of the extract at various concentrations (20, 40, 60, 80, and 100 µg/ml) and 1.0 ml of enzyme solution were mixed together and incubated at 25°C for 10 minutes. After incubation, 1.0 ml of starch (0.5%) solution was added to the mixture and further incubated at 25°C for 10 minutes. The reaction was then stopped by adding 2.0 ml of dinitrosalicylic acid (DNS, color reagent), heated the reaction mixture in a boiling water bath for 5 minutes. After cooling, the absorbance was measured colorimetrically at 565 nm. The inhibition percentage was calculated using the formula:

$$\text{Percentage inhibition} = \frac{\text{Absorbance(control)} - \text{Absorbance(sample)}}{\text{Absorbance(control)}} \times 100$$

Statistical analysis

Data were expressed as mean ± standard deviation.

RESULT AND DISCUSSION

DPPH radical scavenging assay

DPPH radical is used to investigate the antioxidant potential of a given compound *in vitro*. The hydroethanolic leaf extract of *A. catechu* (L.f) Willd at 5 different concentrations (20-100 µg/ml), produced dose-dependent radical scavenging activities. The plant extract showed the maximum scavenging effect on DPPH as 62.94±0.91% inhibition at 100 µg/ml whereas ascorbic acid showed 89.42±0.82% inhibition as shown in the Fig. 1. The IC_{50} value of DPPH radical scavenging activity was found to be 73.81±0.95 µg/ml. The radical scavenging activity indicates the antioxidant capacity of extract and its reducing properties might be associated with the presence of reductones [16]. In a similar study, Sanjay *et al.*, reported that the methanolic heartwood extract of *A. catechu* showed promising DPPH radical scavenging activity. Since the *A. catechu* extract showed DPPH radical scavenging activity, the natural antioxidants present in the extract might possess the ability to neutralize free radicals and thereby act as scavengers, helping prevent cell and tissue damage.

OH^- scavenging assay

OH^- are the most reactive oxygen species that can cause oxidative damage to DNA, lipids, and proteins [17,18]. Scavenging of OH^- is an important antioxidant activity because of very high reactivity of the OH^-, enabling it to react with a wide range of molecules in the living cells, such as amino acids, sugars, lipids, and nucleotides [19]. The maximum scavenging effect for OH^- was obtained at 63.53±1.08% inhibition at 100 µg/ml of the plant extract, whereas the standard ascorbic acid showed 78.14±0.75% inhibition as shown in the Fig. 2. The IC_{50} value of OH^- was found to be 69.03±0.58 µg/ml. The scavenging activity may be due to the presence of flavonoids in the extract. As the hydroethanolic extract of *A. catechu* (L.f) Willd exhibit OH^- activity, it can be assumed that this plant can be used as a marker for oxidative stress in cells and tissues.

NO^- scavenging assay

NO^- causes DNA fragmentation, cell damage, neurotoxicity, and lipid peroxidation [20]. The maximum scavenging effect of nitric oxide was

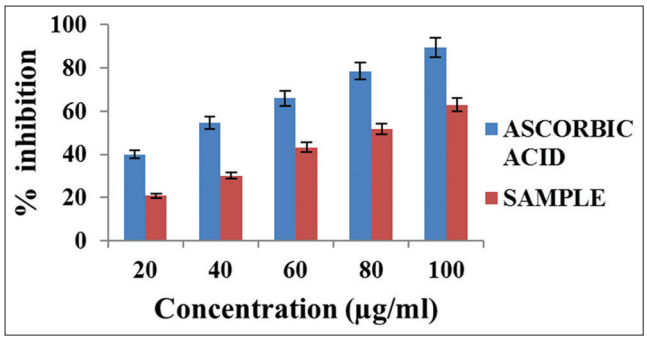

Fig. 1: 1,1-diphenyl-1-picryl hydrazyl radical scavenging assay of *Acacia catechu* (L.f) Willd. leaf extract. Values are represented in mean ± standard deviation of triplicates

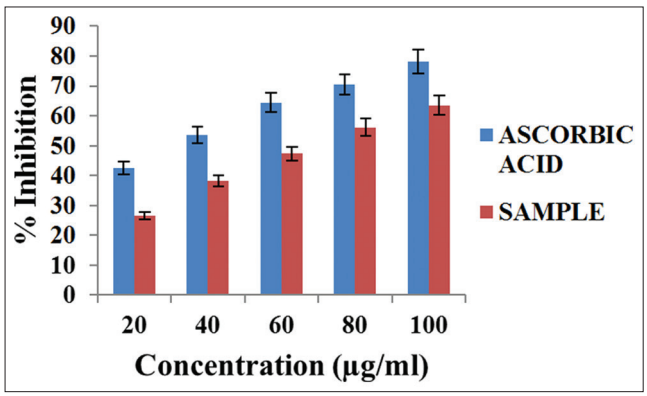

Fig. 2: Hydroxyl radical scavenging assay of *Acacia catechu* (L.f) Willd. leaf extract. Values are represented in mean ± standard deviation of triplicates

obtained at 71.04±0.48% inhibition at 100 µg/ml of the plant extract, whereas the standard ascorbic acid showed 85.78±0.62 % inhibition at the same concentration as shown in the Fig. 3. The IC_{50} value of NO^- was found to be 60.74±0.37 µg/ml. Flavonoids and phenolic compounds may be possibly involved in the NO^- scavenging activity [21]. In a similar study, Bibhabasu *et al.* reported that the 70% methanolic heartwood extract of *A. catechu* (L.f) Willd showed promising NO^- scavenging activity.

SO scavenging assay

Superoxide radical is one of the strongest free radical generated within the living cells [22]. Superoxide anions are well-characterized etiological factors for the pathogenesis of various diseases [23]. The plant extract showed maximum scavenging effect for SO at 51.50±0.86% inhibition at

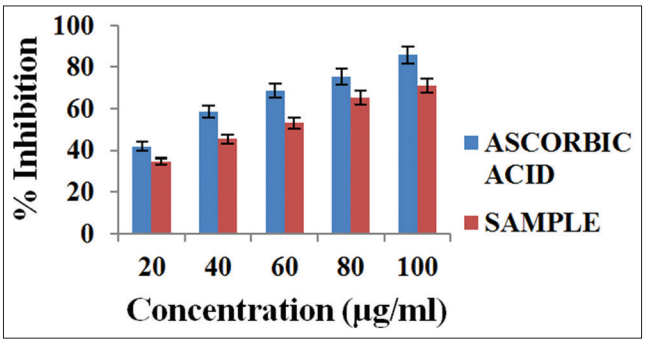

Fig. 3: Nitric oxide radical scavenging assay of *Acacia catechu* (L.f) Willd leaf extract. Values are represented in mean ± standard deviation of triplicates

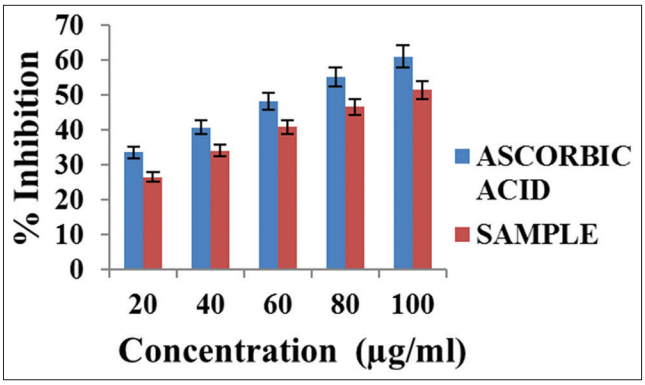

Fig. 4: Superoxide radical scavenging assay of *Acacia catechu* (L.f) Willd leaf extract. Values are represented in mean ± standard deviation of triplicates

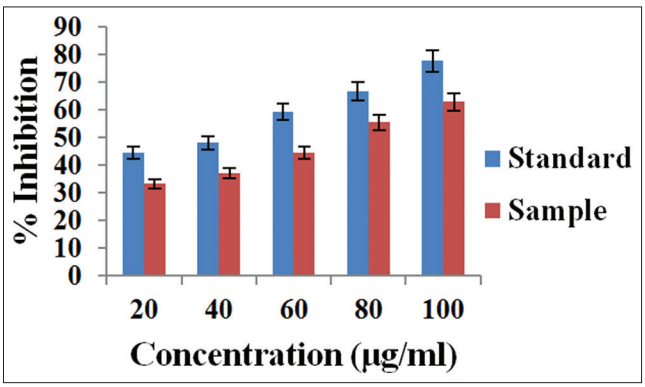

Fig. 5: α-amylase inhibition assay of *Acacia catechu* (L.f) Willd leaf extract. Values are represented in mean ± standard deviation of triplicates

100 µg/ml and 61.11±1.13% was recorded for ascorbic acid as shown in the Fig. 4. The IC_{50} value of SO was found to be 83.10±1.00 µg/ml. The antioxidant property of the secondary metabolites may be attributed to the scavenging of SO. Since the *A. catechu* (L.f) Willd extract showed SO scavenging activity, the extract has the ability to combat various diseases through free radical scavenging activity.

α-amylase inhibition assay

The maximum inhibitory effect for α-amylase by the plant extract was obtained at 62.95% inhibition at 100 µg/ml, whereas the standard maltose showed 77.77% inhibition as shown in the Fig. 5. The IC_{50} value of α-amylase was found to be 71.17 µg/ml. Phenolic compounds are reported to be effective human α-amylase inhibitors [24]. Previous studies on α-amylase inhibitors identified from medicinal herbs recommend that a number of capability inhibitors belong to flavonoid class that has features of inhibiting α-amylase activity [25]. The presence of such inhibitors in food stuff is useful in the control of hyperglycemia as they delay carbohydrate digestion by reducing the rate of glucose absorption, thereby retard the post-prandial plasma glucose rise [26,27].

CONCLUSION

The results obtained from the present study suggest that the hydroethanolic leaf extract of *A. catechu* (L.f) Willd has a significant antioxidant and antihyperglycemic activities and it can be utilized as a potential source of therapeutic agent in the management of diabetes mellitus.

ACKNOWLEDGMENT

Financial support by University Grants Commissions, in the form of Minor Research Project (No.F MRP: 6281/15 [SERO/UGC]) is acknowledged.

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